

STUDIES ON THE EFFECT OF DECOMPRESSION ON
CERTAIN INSECTS, WITH SPECIAL REFERENCE
TO ANOPHELES QUADRIMACULATUS SAY
AND AEDES SOLLICITANS WALKER¹

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INTRODUCTION

Studies on the reaction of insects to varying reduced barometric pressures may lead to a better understanding of their tolerance to high altitudes. This may aid in the determination of the insects of medical importance that can be transported alive in non-pressurized portions of high flying aircraft.

Back and Cotton (1925) studied the effect of a vacuum varying from 26 to 29 inches of mercury on the immature and adult forms of insect pests of stored products at 60 to 70 degrees Fahrenheit. The majority of the adults were killed by an exposure of one day, one species required two days and another four. Stemler and Hiestand (1951) observed that a tolerance to anoxia is formed by insects following repeated, rapid ("explosive") decompression. Working with five orders of insects they found that all adult species showed some tolerance with the second decompression. Tolerance occurred if the interval between the first and second decompression was ten minutes or several hours. Wellington (1946) has studied the reaction of flies at low pressure and simulated storm pressures.

The purpose of the present study was to determine the mortality curve of mosquitoes exposed to decompression at various

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pressure altitudes, as well as observations on their reactions to such treatment.

MATERIALS

The strain of *Anopheles quadrimaculatus* used was obtained through the courtesy of the Orlando Florida Laboratory of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. The colony was reared through many generations before experiments were initiated. The eggs were placed on the surface of tap water containing brewers' yeast and brain heart infusion. Three days after the eggs hatched, the larvae were placed in 8 by 12 inch stainless steel pans, 200 per pan, and fed measured amounts of commercial dog food twice daily for the production of a uniform stock for test purposes. The colony was maintained in a constant temperature room at 85° F. with the humidity at 55 to 60 per cent. The adults were provided undiluted honey in screened Stender dishes, five per cent dextrose solution on cotton wicks, and beginning at the age of three to five days, daily blood meals from a guinea pig. The rearing technique was similar to that of Heal and Pergrin (1945). The ages of the test insects varied from day to day, but similar age groups were used in all tests. It was found that vitality was lost after 14 days and adults above this age were generally unsuitable for tests.

The *Aedes sollicitans* adults were collected in the field and utilized for tests.

PROCEDURE

The adult female mosquitoes were removed from screened cages with a suction tube and placed in an altitude chamber for observation at various simulated pressure altitudes. For observation of the reaction of insects to various altitude levels, the altitude chamber developed by Perry and Webster (1950) was utilized. For extensive mortality tests the equipment was enlarged and modified to provide five chambers which were evacuated by a vacuum pump running continuously. Varied amounts of vacuum were maintained by adjusting an air bleeder valve located between a calibrated gauge and the vacuum pump. By this system a given vacuum could be maintained without variation for eight or more hours.

Ten adult females picked at random from the rearing cage were placed in each of the five chambers and raised rapidly to the desired mercury level. Thirty tests, involving 300 insects, were made for each one-half inch rise of mercury from 19 to 28.5 inches. The tests were run for eight hours at a temperature of $80^{\circ} + -2^{\circ}$. After eight hours the mosquitoes were removed from the chambers by suction tube a few at a time to avoid injury. They were placed in groups of ten in 1,000 ml. beakers covered with 20 mesh copper screen, and fed five per cent dextrose solution in cotton wicks lying over the tops of the screens. The living insects were determined after 16 hours using a criterion of any visible movement of an appendage upon disturbance.

OBSERVATIONS OF REACTIONS OF INSECTS DURING
ASCENTS AND DESCENTS

Aedes sollicitans

In order to observe the reactions of mosquitoes during the ascent to altitude and the subsequent descent, *Aedes sollicitans* was selected because of its relatively large size. Altitudes were measured as inches of Hg vacuum. Five vigorous field collected adult females were placed in the chamber for observation and raised an inch at a time at three-minute intervals to 28.5 inches of mercury. After three minutes at 28.5, they were returned to normal pressure at one-minute intervals. The results are given in Table 1.

Following recompression, the insects were removed from the chamber and placed in a beaker with five per cent dextrose as food. There was no mortality after 16 hours. During the decompression the mosquitoes were apparently normal up to 13 inches of mercury when they became quiet and a slight enlargement of the abdomen was noted. The abdominal distention gradually increased through the steps to 22 inches, at which level it was equal to that of a fully engorged female (Fig. 1). At the highest level, 28.5 inches, all were on their backs and quiet. On the return down to normal the insects began to walk at 21 inches of mercury and to fly at 18. They were apparently normal at the levels from six to zero.

Musca domestica

For comparison, ten six-day old laboratory reared house flies

were treated in exactly the same manner as the *Aedes sollicitans* above (See Table 2).

The flies were apparently normal, flying in approximately the

TABLE 1

REACTIONS OF *Aedes sollicitans* FEMALES DURING ASCENT AND DESCENT

Inches Hg vacuum	Simulated altitude (feet)	Reaction
1-12	1,000-13,571	Normal
13	15,000	Quiet except when vigorously disturbed by tapping. Slight abdominal distention
14-19	16,500-25,400	Occasional flying
20	27,500	Walk when disturbed, no flying
21	29,900	One on feet, others inverted, no walking on disturbance
22-26	32,500-47,250	Quiet with greatly enlarged abdomens, slight leg movement when disturbed
26-28	47,250-62,200	Vigorous leg and wing movement during each change of level
28.5	68,500	All 5 inverted with abdomens greatly distended. Slight leg movement with vigorous tapping of chamber
28	62,200	Leg movements when disturbed
27	53,400	Vigorous movement of legs and wings when disturbed
26	47,250	Flying attempts when disturbed
25	42,500	Two on feet, wing movement on tapping
24	38,600	Five on feet, fly or jump $\frac{1}{4}$ inch in air when disturbed
23	35,400	Five on feet, moderate abdominal distention
22	32,500	Fly or jump $\frac{1}{2}$ inch in air on disturbance
21-19	29,900-25,400	Walk without being disturbed, fly on disturbance
18	23,286	One flew to top of chamber, incoordinated flight in others when disturbed
17-11	21,473-12,143	Walking, attempted flights
10- 9	10,364- 9,455	Normal abdomen, 3 flew $\frac{1}{2}$ inch on disturbance
8- 7	8,375- 7,222	Attempts at flight, one flew to top of chamber
6- 0	6,111- 0	All 5 apparently normal, abdomens normal

same ranges as the mosquitoes (0-21 inches in mercury). There was no visible distention of the abdomens. At 28.5 inches Hg there was no movement visible and this remained true on the way

down to 26 inches when the first movement was observed. The insects began righting themselves at 24 inches and all were on their feet by 17 inches. All were apparently normal from 15 inches on down. Sixteen hours after exposure, one fly was dead while the controls remained normal.

It is evident from these tests that both *Aedes sollicitans* and *Musca domestica* females can withstand gradual decompression



Fig. 1. Mosquitoes under decompression (note distended abdomens).

to 28.5 inches of mercury and return to normal pressure without ill effects, that they seem to be not adversely affected by the ascent to from 19 to 21 inches, are relatively quiet at higher levels, and that the mosquitoes acquire greatly distended abdomens at extremely low pressures. The two insects vary in ability to withstand a high vacuum sustained over a period of time. The flies were found to withstand 28.5 inches of mercury for one hour with no mortality 16 hours later. However, when ten field collected *Aedes sollicitans* were raised within 15 seconds to 28.5

inches of mercury for 30 minutes, then dropped suddenly to normal, 70 per cent were dead, 16 hours after exposure, while the controls remained normal. Within three minutes after decompression, all were quiet except for the slight movement of wings on one insect, and all abdomens were greatly distended. Upon

TABLE 2

REACTIONS OF *Musca domestica* ADULTS DURING ASCENT AND DESCENT

Inches Hg vacuum	Simulated altitude (feet)	Reaction
1-15	1,000-18,000	Apparently normal, walking and flying
16-21	19,800-29,900	Quiet, preening wings, flying on disturbance
22	32,500	Quiet, 4 preening wings, do not fly on disturbance
23	35,400	Three lying flat on ventral portion of thorax, 1 flew when disturbed
24	38,600	One rubbing legs together, 2 attempting to fly, remainder, no noticeable movement on disturbance
25	42,500	One walking, 4 moving legs
26	47,250	Mouthparts moving in 1, no noticeable movement in remainder
27-28.5	53,400-68,500	No movement on disturbance
28-27	62,200-53,400	No movement
26-25	47,250-42,500	One wing moved, right and left rear legs moved on 1
24	38,600	One moving legs, one upright on feet
23-21	35,400-29,900	Two on feet rubbing legs together
20	27,500	Six on feet preening wings
18	23,286	Eight on feet
17	21,470	Ten on feet preening heads and wings
16	19,800	One flew to top of chamber
15- 0	18,000- 0	Normal

sudden recompression to normal after 30 minutes, movement was noted in two insects immediately, but it was 14 minutes before five moved and two attempted flights. During one-hour exposure under the same conditions, there was great activity during decompression, but all were quiet in one minute. No movement was noted for six minutes when slight leg and proboscis movements were seen. At 14 minutes convulsive movements were seen

in one mosquito. There was no movement immediately upon return to normal. Leg motion was noted in two insects after two minutes. Sixteen hours later, all were dead.

ALTITUDE-MORTALITY DATA

Anopheles quadrimaculatus

Having studied the visible effect of decompression on mosquitoes and flies, the altitude-mortality curve was determined for *Anopheles quadrimaculatus*. This species was chosen because of its connection with disease transmission. A constant exposure time of eight hours was chosen because initial tests indicated that the insects could withstand considerable decompression over this period of time. The gauge on the altitude chamber was calibrated with a mercury manometer, and the estimated altitude computed from the Standard Atmosphere Tables of Diehl (1925). Exposures were made for each one-half inch rise in mercury level from 19 to 28.5 inches. The results were combined for each one-inch rise for the data shown in Table 3.

TABLE 3
TOLERANCE OF *Anopheles quadrimaculatus* TO DECOMPRESSION
3,014 FEMALES, 8 HOURS' EXPOSURE

Inches Hg vacuum	Simulated altitude (feet)	Per cent dead
19-19.5	25,400-26,440	17 ± 3
20-20.5	27,500-28,700	27 ± 4
21-21.5	29,900-31,200	37 ± 5
22-22.5	32,500-33,800	40 ± 5
23-23.5	35,400-36,900	46 ± 7
24-24.5	38,600-40,460	56 ± 8
25-25.5	42,500-44,700	71 ± 5
26-26.5	47,250-50,100	87 ± 5
27-27.5	53,400-57,400	96 ± 2
28-28.5	62,200-68,500	100
Controls	Sea Level	3 ± .1

Three hundred tests averaging ten insects each were conducted (30 for each combined one-inch mercury level) utilizing a total of 3,014 insects. The controls contained 73 units of ten insects each. Mortality based on the vast visible movement of an insect's

appendage 16 hours after exposure, ranged from 17 per cent at 19–19.5 inches of mercury to 100 at 28–28.5 inches. From the smooth curve, the 50 per cent mortality point occurred at 36,800 feet corresponding to approximately 23.5 inches of vacuum. The altitude mortality curve is shown in Figure 2. On the basis of recovery over a period of 24 hours, however, 100 per cent mortality occurred at 26.5–27 inches of mercury with 98 per cent mortality occurring at 26 inches. It is therefore evident that very little ultimate survival occurs following eight hours' decompression above this level.

Since 26 inches of mercury appears to be the critical level for total mortality, ten *Anopheles quadrimaculatus* females, ten days old, were observed at this level for eight hours to determine their cumulative reactions. A summary of these observations is given in Table 4.

Sixteen hours after exposure all were dead. The ten controls were normal. Table 4 shows that the mosquitoes retained some

TABLE 4
REACTION OF *Anopheles quadrimaculatus* TO DECOMPRESSION
(26 INCHES HG VACUUM)

Time (minutes)	Reaction
1	Very ataxic immediately following decompression
15–75	Two active, opening and closing wings rapidly, ataxic, one flew across chamber
105	All quiet, one on back, all active when disturbed
135	Three on backs, remainder very ataxic but active
165–255	Five on backs, others quiet, unable to walk when disturbed
285–345	Six to nine on backs, one attempted walking and flying, ataxic
375–435	Nine to ten on backs, slight leg movement when disturbed
465–480	Ten on backs, no movement
480	Vacuum released, one righted itself immediately, one flew around chamber when disturbed, one moving legs, others immobile

movement for seven-and-a-quarter hours at 26 inches of mercury vacuum and two were apparently normal immediately after exposure. However, they were apparently sufficiently affected to cause death in 16 hours. Subsequent tests (30, involving 300 insects) at 26–26.5 inches of mercury indicated that this single

test is not a true indication of mortality. The greater number of tests showed 87 per cent mortality in 16 hours increasing to 98 per cent in 24 hours.

Aedes sollicitans

An altitude mortality test was run on *Aedes sollicitans* adult females under the same conditions described above for *Anopheles quadrimaculatus* except one-inch mercury intervals were used. This test was for comparison only and did not involve as many individuals (370 insects, 37 chambers of ten each). The results are given in Table 5 and shown diagrammatically in Fig. 3.

TABLE 5
TOLERANCE OF *Aedes sollicitans* TO DECOMPRESSION
370 FEMALES, 8 HOURS' EXPOSURE

Inches Hg vacuum	Simulated altitude (feet)	Number females	Per cent dead
18.5	24,400	50	6 ± 6
21	29,900	50	62 ± 10
22	32,500	50	70 ± 7
23	35,400	50	80 ± 7
24	38,600	30	87 ± 6
25	42,500	50	92 ± 4
26	47,250	50	94 ± 3
26.5	50,100	40	100
Controls	Sea Level	82	9 ± 3

These data indicate that *Aedes sollicitans* reaches 100 per cent mortality at 26.5 inches of mercury 16 hours after exposure. *Anopheles quadrimaculatus* reached this point 24 hours after exposure to 26.5–27 inches of mercury. It is probable that there is very little difference in the tolerance of these two species. With reference to simulated altitudes, it will be necessary to expose the insects to the approximate temperatures inherent at the various levels. The extreme temperatures at the high altitudes may lower the tolerance level considerably. On the other hand, lowered metabolism may somewhat increase tolerance.

Culex restuans

Field collected *Culex restuans* were exposed to decompression at 26 inches of mercury for eight hours at 78° F. Ten chambers

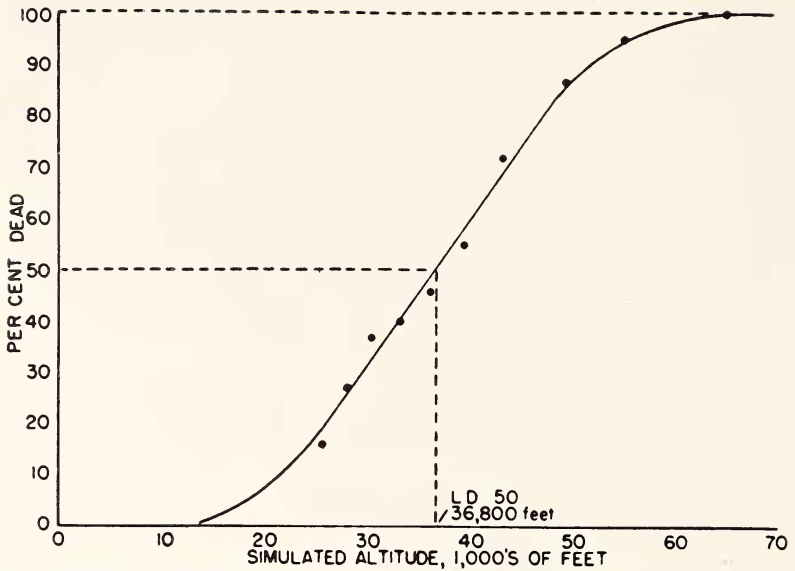


FIGURE 2 Altitude-Mortality Curve for *Anopheles quadrimaculatus* 8 hour exposure.

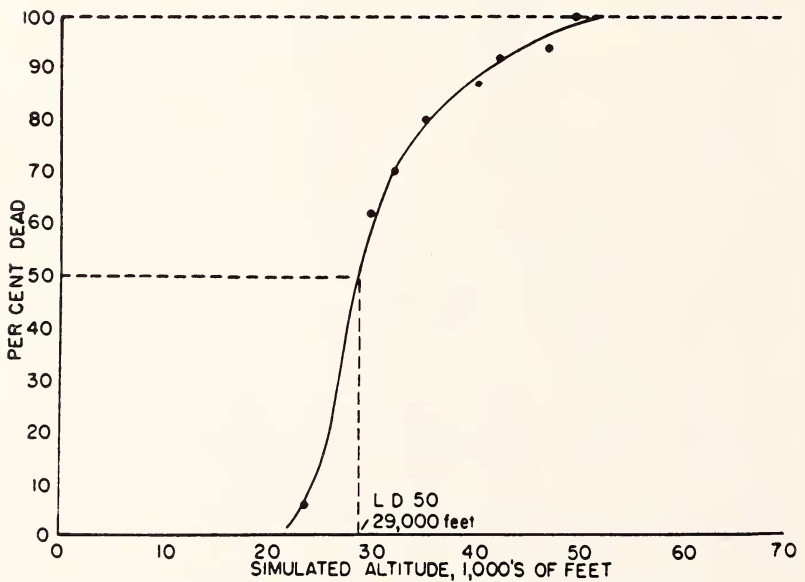


Figure 3 Altitude-Mortality Curve for *Aedes sollicitans* 8 hour exposure

were utilized averaging 11 females per chamber. One hundred per cent mortality was attained 16 hours after completion of the exposure with three per cent mortality in the controls. This indicates that *Culex restuans* probably does not withstand decompression greater than the critical level for *Anopheles quadrimaculatus* and *Aedes sollicitans*. Sufficient tests were not made to give more than an indication on the tolerance of this species and the critical mortality level could conceivably be much lower.

SUMMARY

Observation of *Aedes sollicitans* adult females at various ascending levels of decompression at room temperature showed gradual abdominal distention ultimately equaling that of a fully engorged female at 22 inches of mercury (32,500 feet). The insects were immobile at 28.5 inches (68,500 feet) and inverted. On descending to normal, they began to walk at 21 inches of mercury (29,900 feet), to fly at 18 (23,280 feet), and were apparently normal at levels from six inches (6,100 feet) to zero.

Houseflies acquired no abdominal distention at 28.5 inches of mercury (68,500 feet), were immobile from this level down to 26 inches (47,250 feet), were on their feet at 17 inches (21,470 feet), and were apparently normal from 15 inches (18,000 feet) down. Both the mosquitoes and flies withstood temporary decompression to the above level without apparent injury. The flies withstood 28.5 inches of mercury (68,500 feet) for one hour, while after 30 minutes exposure there was 78 per cent mortality in the mosquitoes.

Studies on the tolerance of *Anopheles quadrimaculatus* to eight hours' exposure at various mercury levels indicated that at room temperature 100 per cent mortality occurred at 28 inches (62,200 feet), based on the visible movements of appendages 16 hours after exposure, and based on recovery after 24 hours, at 26.5–27 inches (50,100–53,400). *Aedes sollicitans* reached 100 per cent mortality at 26.5 inches (50,100 feet) indicating that there is probably very little difference in the tolerance of the two species of mosquitoes. It is possible that decompression to various levels together with the extremely low temperatures found at high altitudes may lower the tolerance level. On the other hand the resulting low metabolism may have a tendency to increase tolerance.

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Minutes of meetings of May 5, 1953 and May 19, 1953 are not available.

MEETING OF OCTOBER 6, 1953

A regular meeting of the Society was held at the American Museum of Natural History. President Clausen was in the chair. There were seven members and four guests present.

Dr. Marion R. Smith of the Agricultural Research Service, Dept. of Agriculture, Washington 25, D.C. was proposed for membership. On motion duly made and seconded, the By-Laws were suspended and Dr. Smith was duly elected.

The meeting was devoted to the summer activities of the members.

Dr. Clausen mentioned that Dr. Klots had been to Europe during the past summer and that he would be a speaker at some future date. She also mentioned a visit to Mrs. Mutchler who was much impressed by the article on Mr. Mutchler in a recent issue of the *Journal*.

Dr. Vishniac called attention to the fact that his son, DeWolf Vishniac, had made some significant contributions to the mechanisms of photosynthesis. Dr. Vishniac then treated the Society to a description of his own activities both in the entomological and non-entomological fields of interest. With monotonous regularity it seems that Dr. Vishniac is called on to do the impossible. Among other things he was asked to photograph the facial expression of a dying mosquito. The high point of the evening was his description of the conflict between a reluctant sea bass and a squid frequently requiring artificial respiration. The sea bass ate the squid—the squid discharged its "ink"—the only victor, triumphant and unsullied was—Dr. Vishniac.

Dr. Pohl spoke about fine collections of Coleoptera he had seen exhibited in France. At an exhibition there, he had also seen an excellent display of Lepidoptera of Madagascar.

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